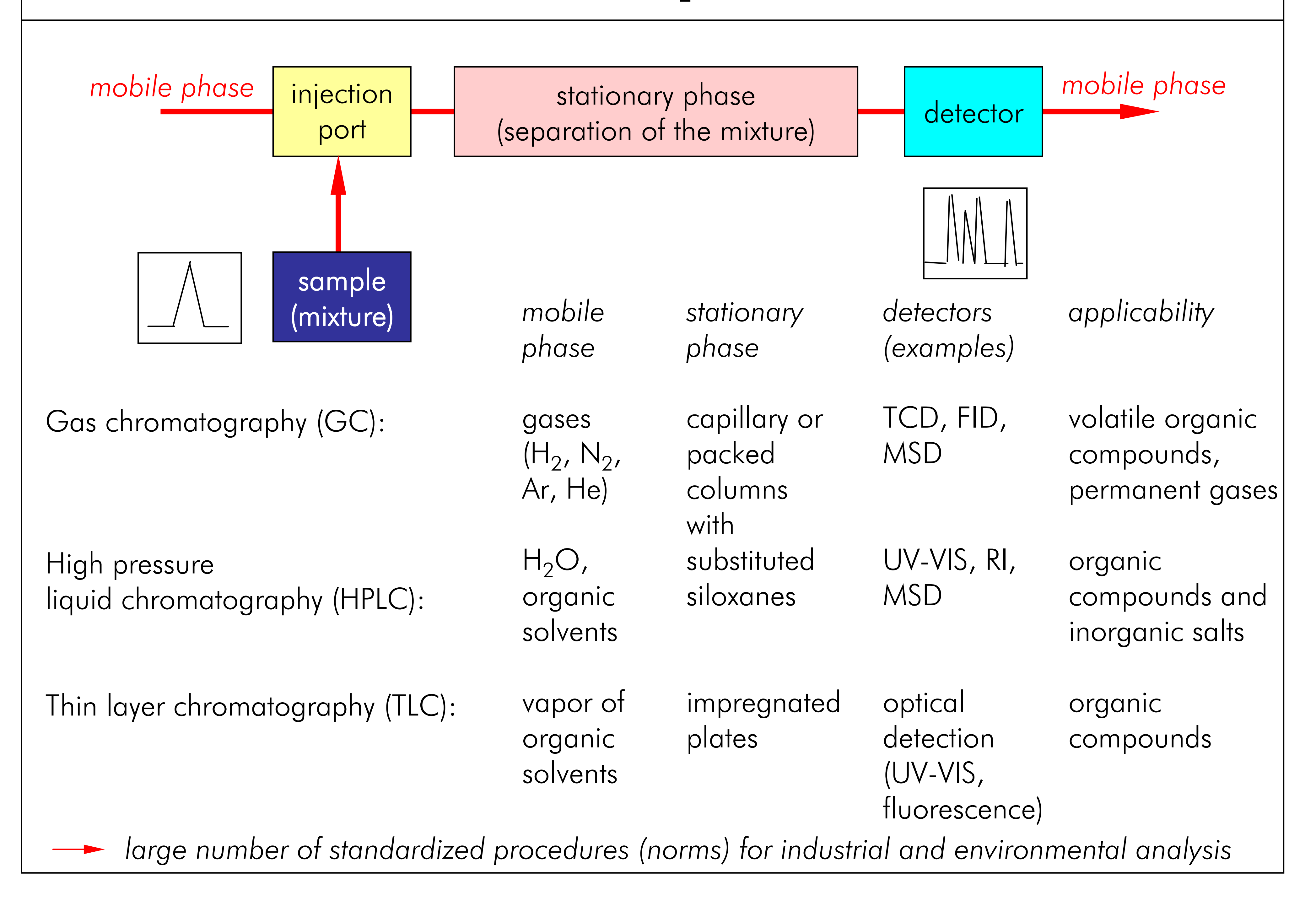
Course „Modern Analytical Methods in Chemical Industry“

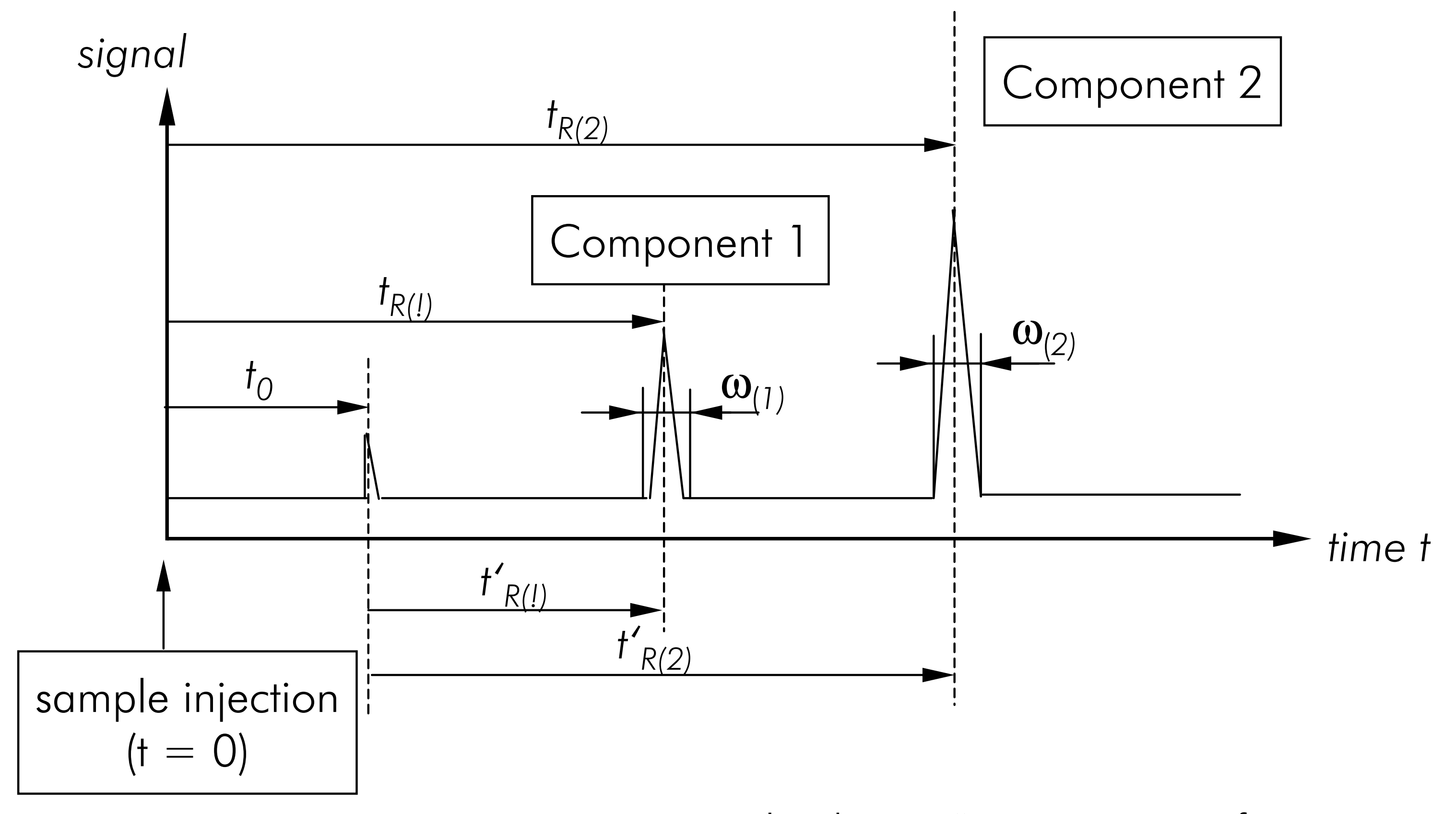
Chromatographic Techniques

Principle



mobile phase
injection port
stationary phase (separation of the mixture)
detector
mobile phase
sample (mixture)
mobile phase
stationary phase
detectors (examples)
applicability
Gas chromatography (GC):
gases (H_2, N_2, Ar, He)
capillary or packed columns with substituted siloxanes
TCD, FID, MSD
volatile organic compounds, permanent gases
High pressure liquid chromatography (HPLC):
H_2O, organic solvents
UV-VIS, RI, MSD
organic compounds and inorganic salts
Thin layer chromatography (TLC):
vapor of organic solvents
impregnated plates
optical detection (UV-VIS, fluorescence)
organic compounds
large number of standardized procedures (norms) for industrial and environmental analysis

Fundamentals of Chromatography



t_0 - „death time“ = retention of a compound with no interaction with the stationary phase

$t_{R(1)}$ - total retention time of component 1

$t_{R(2)}$ - total retention time of component 2

$t'_{R(1)}$ - net retention time of component 1 ($= t_{R(1)} - t_0$)

$t'_{R(2)}$ - net retention time of component 2 ($= t_{R(2)} - t_0$)

$\omega_{(1)}$ - base peak with of component 1

$\omega_{(2)}$ - base peak with of component 2

Equations in Chromatography (1)

Capacity factor k'
(ratio between the residence times of the sample in the stationary and the mobile phase)

$$k' = \frac{t'_R}{t_0} = \frac{t_R - t_0}{t_0} = \frac{t_R}{t_0} - 1$$

Relative retention/Separation factor/Selectivity α

$$\alpha = \frac{k'_{(2)}}{k'_{(1)}}$$

→ Separation of two components is only possible if $\alpha > 1$

Linear velocity u

$$u = \frac{L}{t_0} = \frac{F}{q}$$

L - length of the column
t_0 - dead time
F - Feed ratio of the mobile phase [ml/s]
q - free cross-sectional area of the column

Equations in Chromatography (2)

Porosity of a column ε_T

$$\varepsilon_T = \frac{q}{\pi \cdot r^2} = \frac{F \cdot t_0}{\pi \cdot r^2 \cdot L} = \frac{F \cdot t_0}{V_R}$$

r - radius of the column
V_R - volume of the empty column

Permeability K

$$K = \frac{u \cdot L}{\Delta p} = \frac{L^2}{\Delta p \cdot t_0}$$

Δp - pressure difference between inlet and outlet of a column

→ large K - wrong particle packing, low K - clogging

Specific permeability K^0

$$K^0 = K \cdot \eta \cdot \varepsilon_T = \frac{d_p}{1000}$$

η - viscosity of the mobile phase
d_p - particle diameter of the packing

Equations in Chromatography (3)

Peak broadening

$$N = \frac{16}{(t_{R(i)} \cdot \varpi_{(i)})^2} = \frac{5.54}{(t_{R(i)} \cdot \varpi_{0.5,(i)})^2}$$

N - number of plates
ω - base peak with
$\omega_{0.5}$- peak with at half peak height

$$H = \frac{L}{N}$$

H - height of plates

Effective number of plates $N_{eff.}$ (independent on components of the sample)

$$N_{eff.} = N \cdot \left(\frac{k'}{k'+1} \right)^2 = 16 \cdot \left(\frac{t'_R}{\varpi} \right)^2$$

Equations in Chromatography (4)

Resolution R between 2 peaks

$$R = 2 \cdot \frac{t'_{R(2)} - t'_{R(1)}}{\varpi_{(1)} + \varpi_{(2)}} \approx \frac{\Delta t'}{\varpi}$$

(for peaks close one to the another)

$$R = \frac{1}{4} \cdot \frac{(\alpha - 1)}{\alpha} \cdot \frac{k'_{(2)}}{k'_{(2)} + 1} \cdot \sqrt{N}$$

$(\alpha - 1)/\alpha$ - selectivity term (determined by stationary and mobile phases)

$k'_{(2)}/(k'_{(2)} + 1)$ - capacity term (only significant for $0 < k'_{(2)} < 5$, for large $k'_{(2)} \rightarrow 1$)

$N^{1/2}$ - effectivity term (determined by d_p, L and u)

→ R = 1 → 98 % peak separation, R = 1.5 → fully separated peaks

Equations in Chromatography (5)

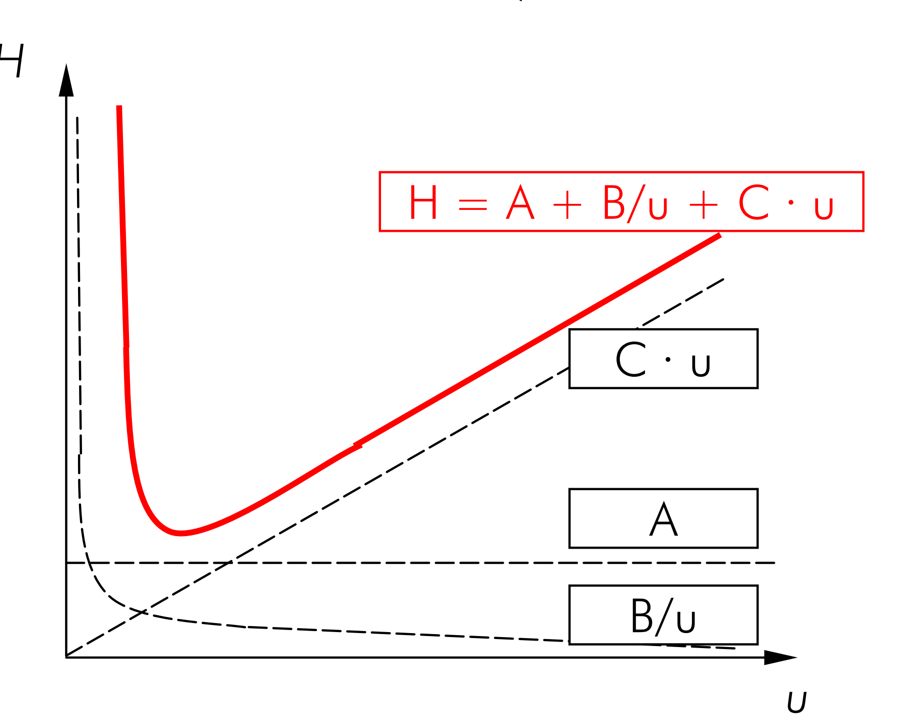
Van Deemter equation

$$H = A + \frac{B}{u} + C \cdot u$$

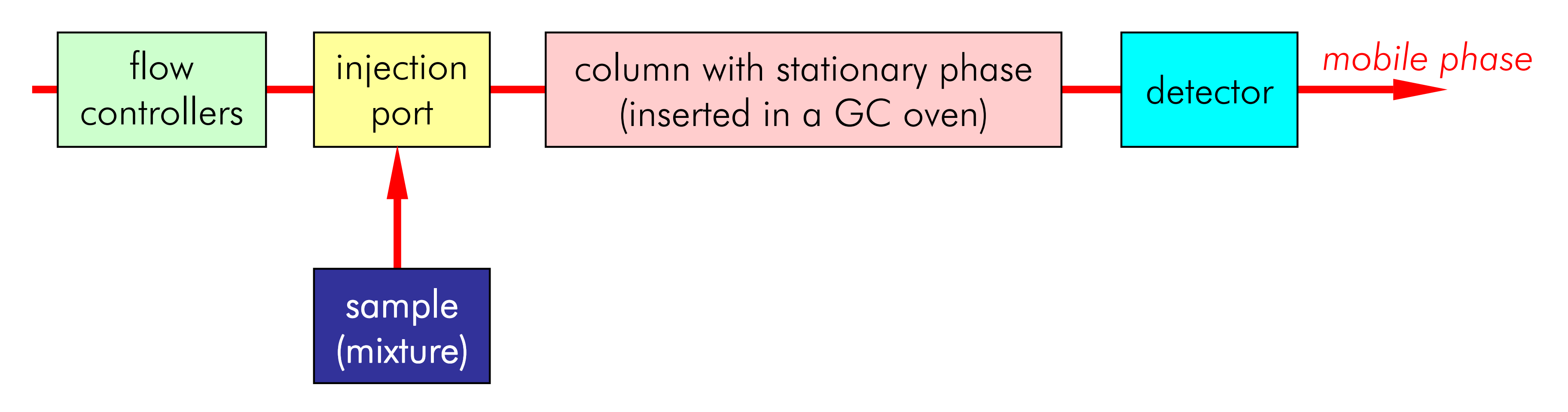
Eddy diffusion term (peak broadening by different ways of sample molecules in a column)

axial diffusion term (statistic axial distribution of sample molecules)

term for mass exchange between stationary and mobile phases (adsorption/desorption, diffusion/back diffusion)



Gas Chromatography



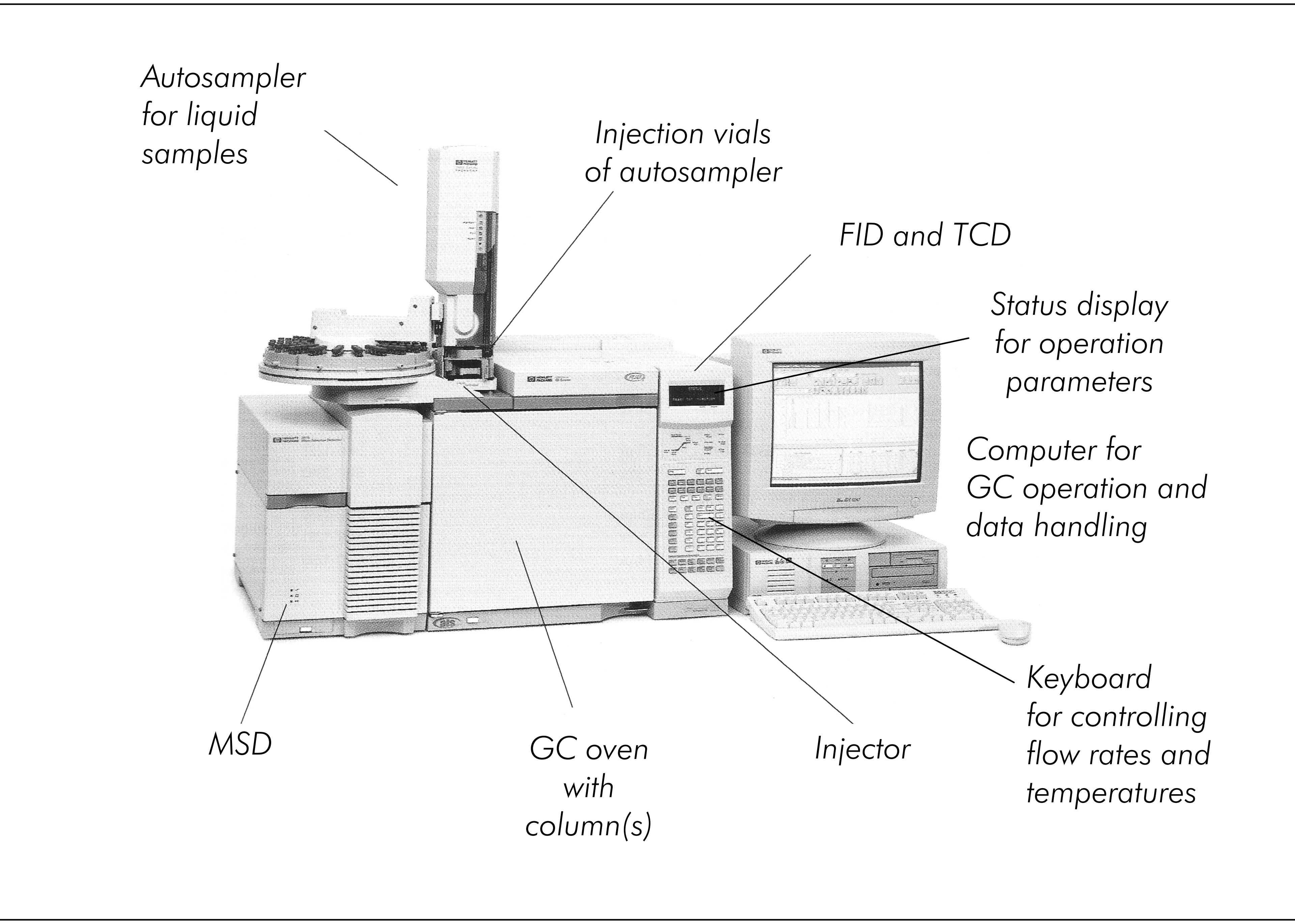
Samples: - gaseous and liquid samples
- limitations: compound which should be analyzed, should be stable under GC operation conditions and should have a vapor pressure significantly higher than zero
- sample preparation: filtration, extraction, if necessary derivatisation (= conversion of „critical“ substances to such with higher stability and vapor pressure, e.g. carbon acids to esters)

Mobile phases:
- He, H_2, Ar, N_2 (purity 99.999 % or better)

Duration of an analysis:
- 5 ... 60 min (standard GC), < 2 min (Micro GC)

Application: - purity control, quality management and certification (wide application)
- environmental and pharmaceutical analysis
- analysis of main and trace components (% to ppm)

GC Setup



Autosampler for liquid samples
Injection vials of autosampler
FID and TCD
Status display for operation parameters
Computer for GC operation and data handling
MSD
GC oven with column(s)
Injector
Keyboard for controlling flow rates and temperatures

Sample Injection

Liquid samples:

- manual (using a syringe, 0.1 – 10 μl, identification of compounds)
- automated by autosampler (need of 1 - 2 ml sample solution, high reproducibility, use for quantitative analysis)
- "on column" injection for samples with low thermal stability (injection directly on top of the cold column, than slow heating) → high precision, but danger of column overloading and pollution
- temperature programmed vaporization – PTV (injection into the cold injector, than temperature programmed heating and vaporization) → high sensitivity and reproducibility, protection of the column
- "head space" injection - sampling of the vapor phase over the sample (useful if the sample contains solid particles)

Gaseous samples

- manual (using a gas-dense syringe, 5 – 50 μl)
- automated by gas sampling valves (0.25 - 5 ml)

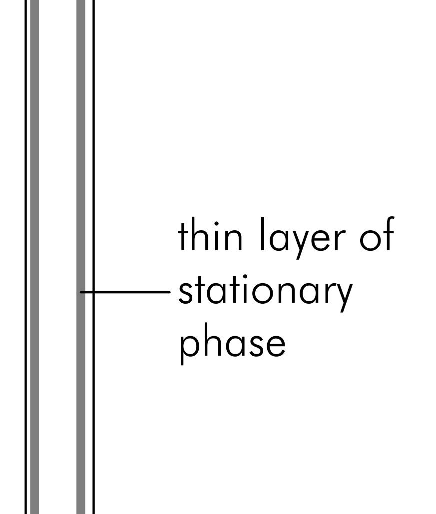
Injector types:

- split-splitless injector
- volatile interface

GC Columns

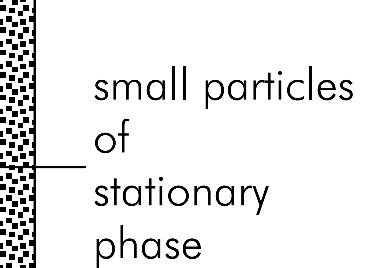


Capillary columns



d = 0.1 – 0.53 mm,
l =10 – 50 m,
film thickness:
0.3 – 50 μm

Packed columns



d = 0.53 mm,
l =0.5 – 10 cm,
particle diameter:
45 – 120 mesh

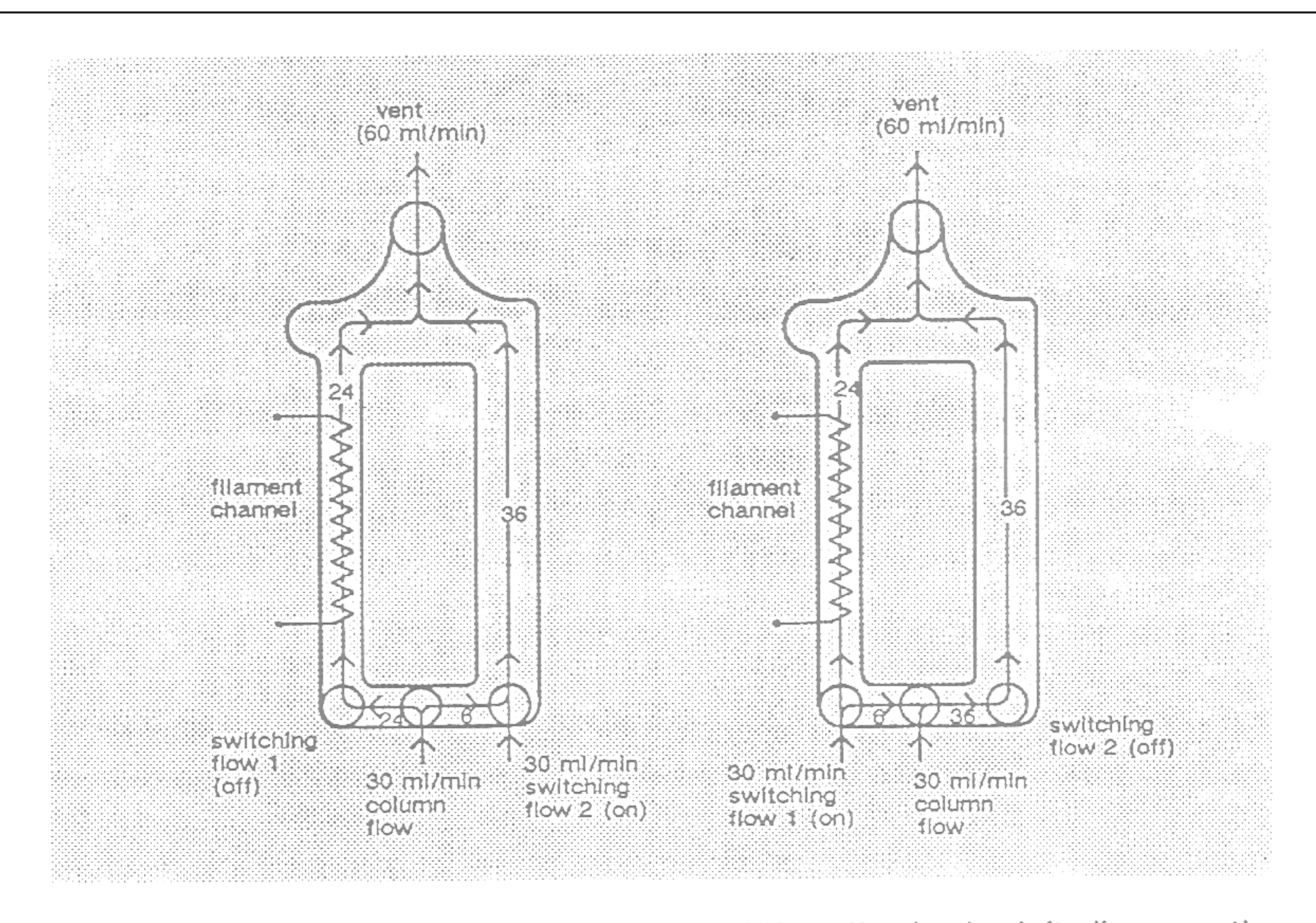
Retention behavior depends on:
- polarity of sample and stationary phase
- volatility of the sample compound
- velocity of the carrier gas (mobile phase)
- temperature
  → temperature programs

Stationary phases
- silicon polymers (polysiloxanes, Si-O-R)
  R = methyl – non-polar,
  R = phenyl or cyanopropyl – intermediate polarity,
  R = ethylene glycol or fluorinated hydrocarbon – polar,
  variation of polarity by co-polymerization)
- PLOT phases (porous layer open tubular)
  small particles immobilized in the wall,
  for separation of high volatile compounds,
  typical stationary phases:
  Al_2O_3, molsieve 5A,
  polystyrene-divinylbenzene (DVB)
- Selected column manufactures:
  J & W (Agilent),
  Chrompack, Restek, Supelco

GC Detectors

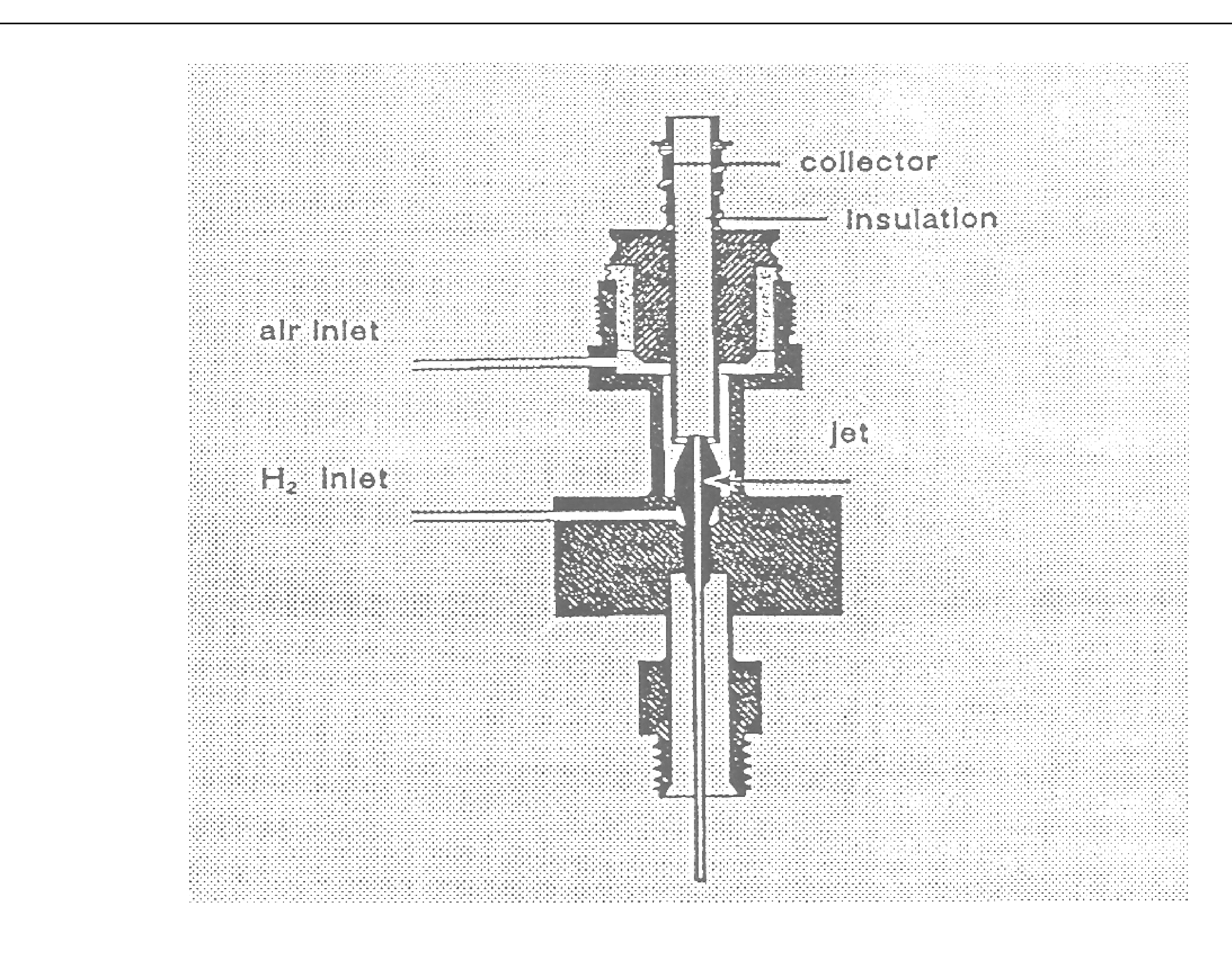
Name	*Selectivity*	*Typical minimum detectable level*	*Linear dynamic range*
TCD Thermal conductivity detector	non-selective (anything what differs from the carrier gas)	400 pg/ml carrier	10^6
FID Flame ionization detector	materials that are ionized in air/H_2 flames (e.g. hydrocarbons)	5 pg C/s	10^7
MSD Mass selective detector	tunable for any species	10 ng (SCAN) 10 pg (SIM)	10^5
ECD Electron capture detector	halogens	0.1 pg Cl/s	10^4
NPD Nitrogen phosphorus detector (Thermoionic detector)	N, P, heteroatoms	0.4 pg N/s 0.2 pg P/s	10^4
FTIRD Fourier transformed infrared detector	molecular vibrations (e.g. organic compounds)	>1 ng (depending on absorption)	10^3
AED Atom emission detector	tunable for any element	0.1 – 20 pg/s (depending on the element)	10^4

Thermal Conductivity Detector (TCD)

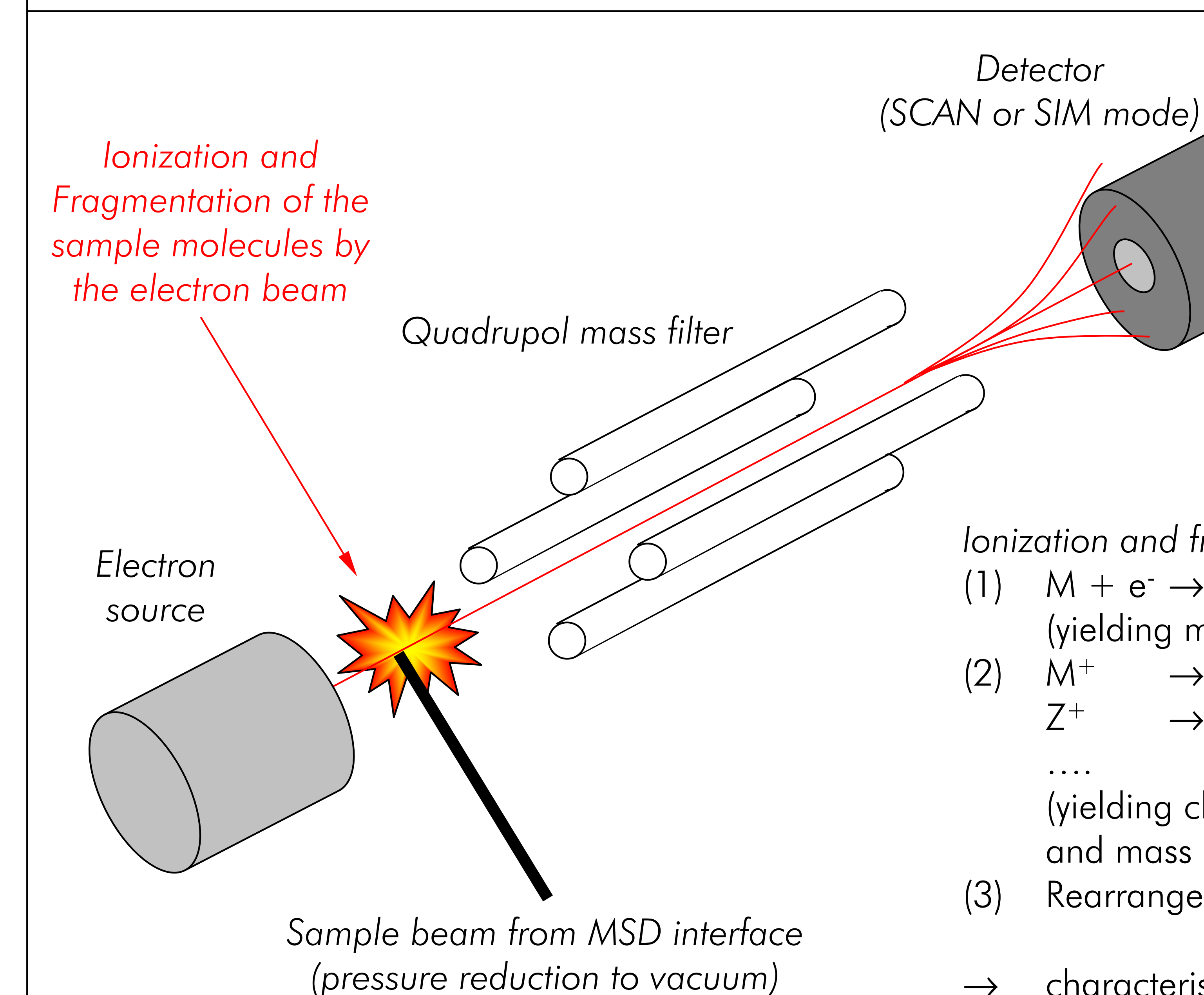


Flow diagram of a commercially available TCD cell. In the left diagram, the switching flow causes the column effluent to pass through the filament channel. When the switching flow changes (right diagram), the column effluent will pass through the empty channel. During this time the filament channel fills with the switching gas, and reference measurements are made. Switching between the column effluent and reference gas occurs every 100 milliseconds.

Flame Ionization Detector (FID)



Mass Selective Detector (MSD)



Ionization and fragmentation:

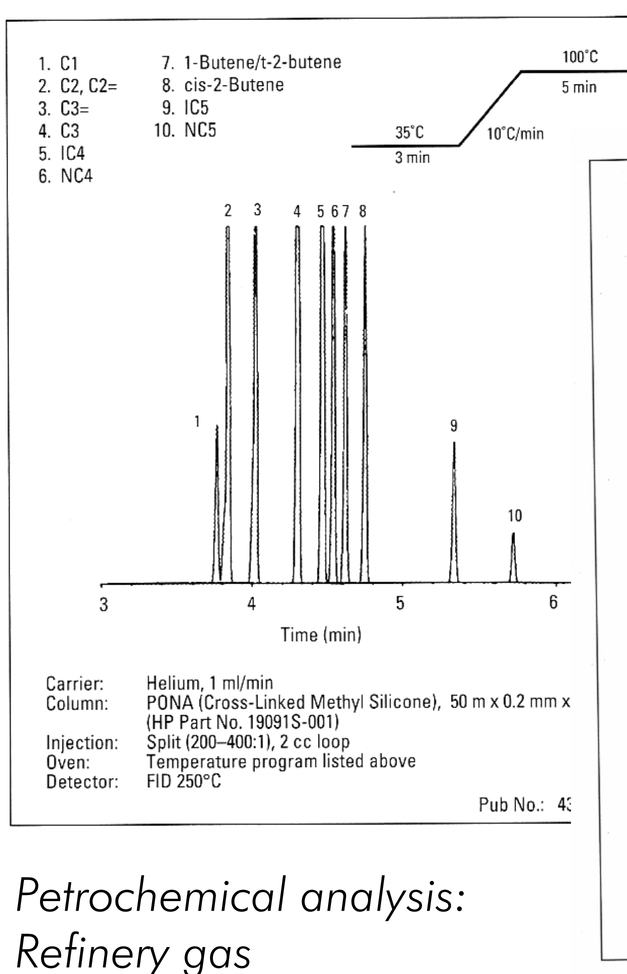
(1) $M + e^- \rightarrow M^+ + 2\,e^-$
(yielding molar peak M^+)

(2) $M^+ \rightarrow Z^+ + (M - Z)$
$Z^+ \rightarrow Y^+ + (Z – Y)$
....
(yielding characteristic mass peaks and mass differences)

(3) Rearrangement reactions

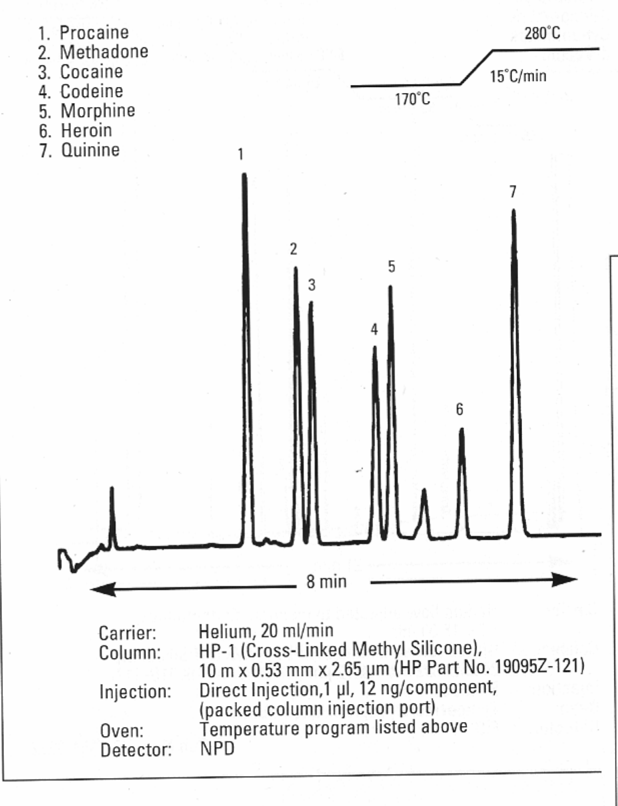
→ characteristic mass spectra, identification of substances possible

GC Application Examples

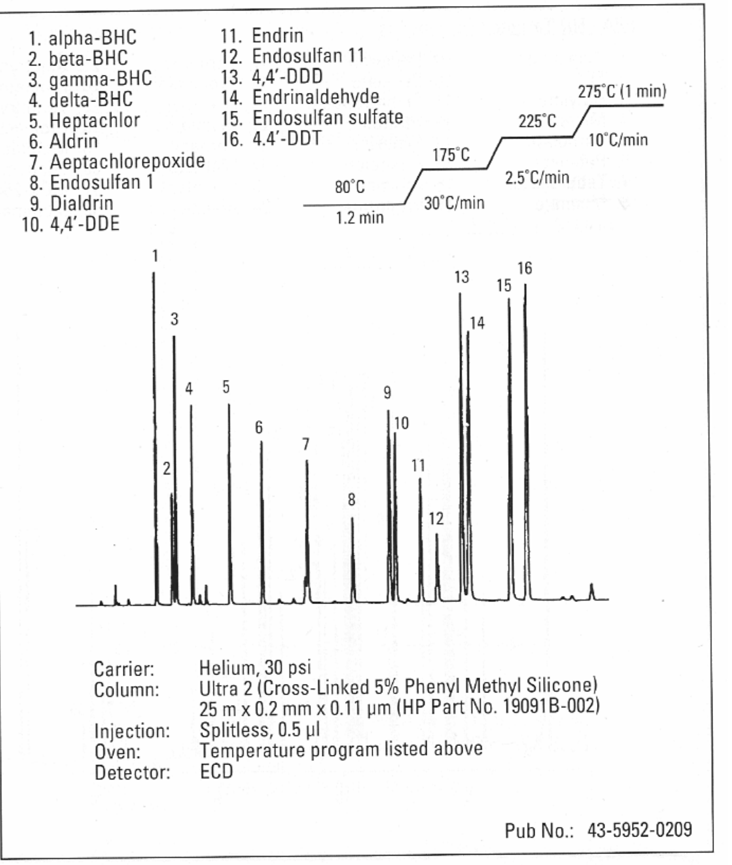


Petrochemical analysis: Refinery gas

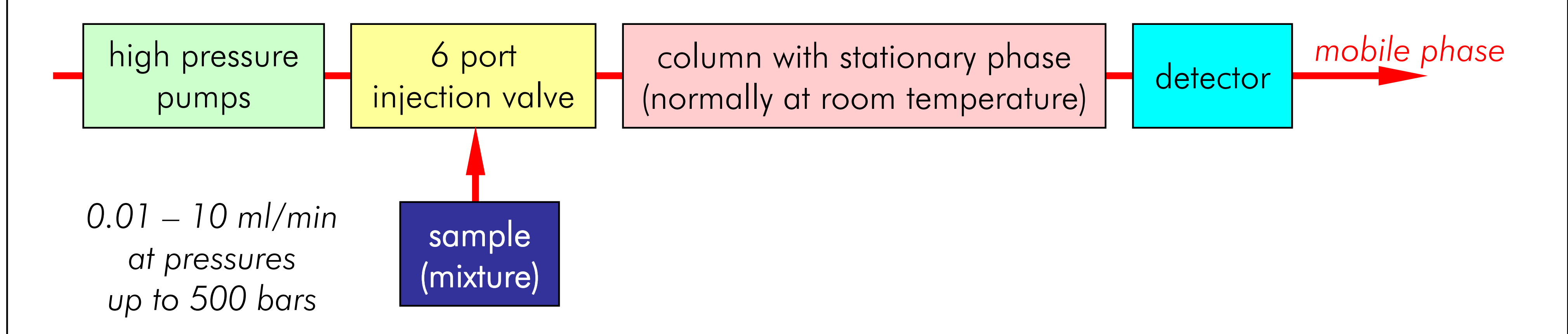
Pharmaceutical analysis: Alkaloid street drugs



Environmental analysis: Chlorinated pesticides



High Pressure Liquid Chromatography (HPLC)



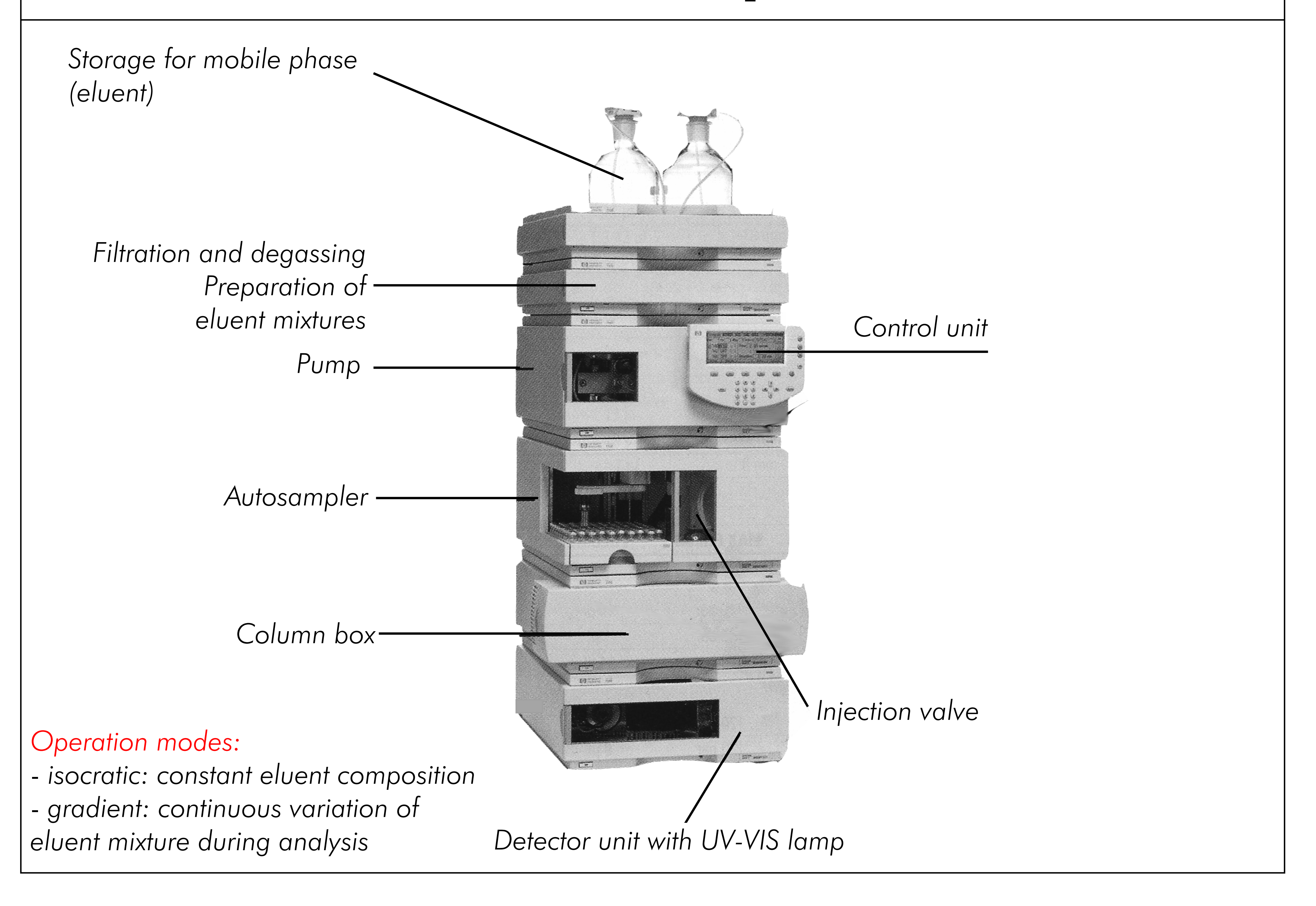
Samples:
- liquid samples
- limitations: solubility in the mobile phase, no thermal restrictions
- sample preparation: filtration, extraction

Duration of an analysis:
- 5 ... 60 min

Application:
- purity control, quality management and certification (wide application)
- environmental and pharmaceutical analysis
- analysis of main and trace components (% to ppm)

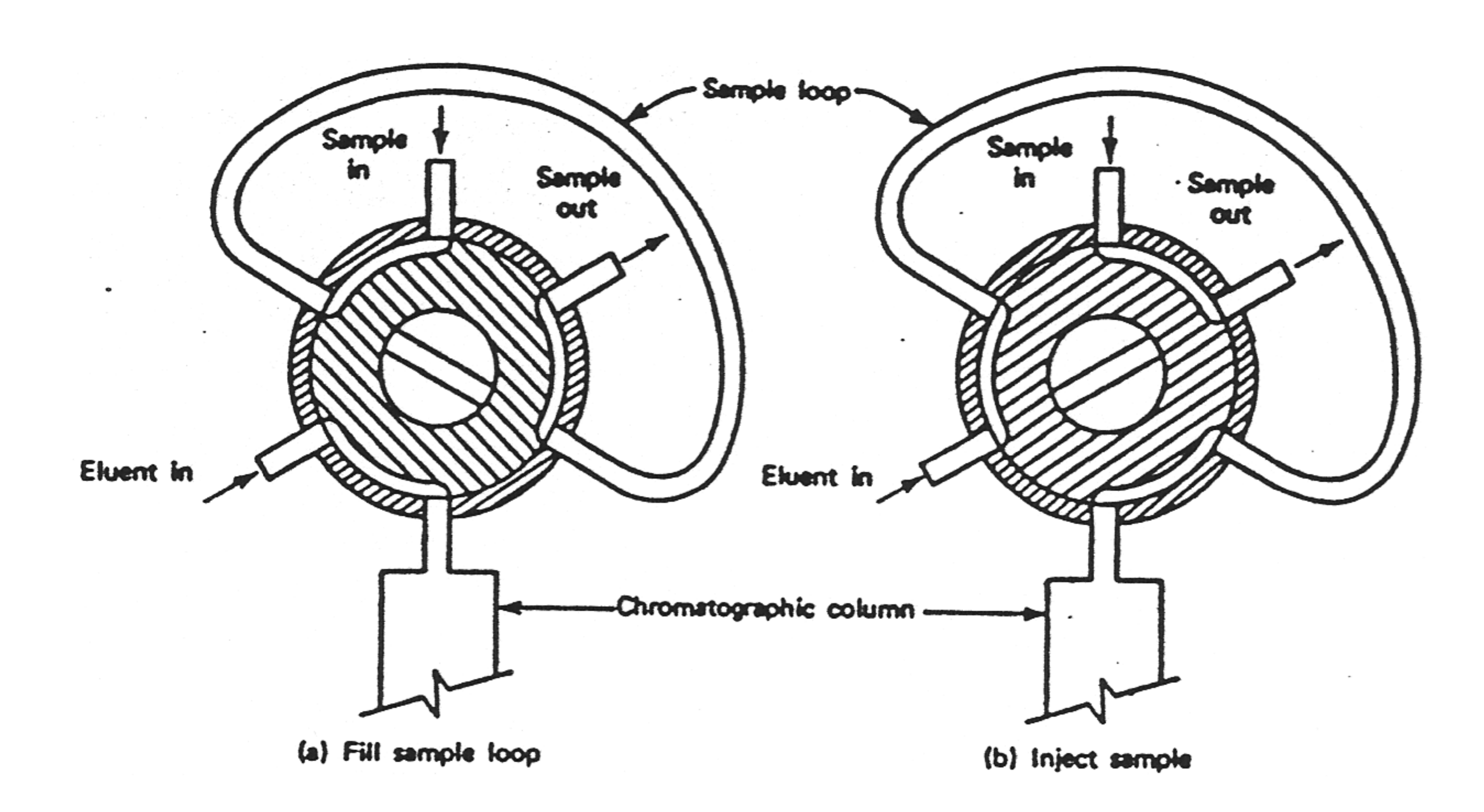
	Mobile Phase	*Stationary Phase*
Normal Phase HPLC	non-polar (e.g. hydrocarbons)	polar
Reversed Phase HPLC	polar (H_2O, buffer solutions, alcohols, acetonitrile and mixtures of them)	non-polar

HPLC Setup


Storage for mobile phase (eluent)
Filtration and degassing
Preparation of eluent mixtures
Pump
Control unit
Autosampler
Column box
Injection valve
Detector unit with UV-VIS lamp
Operation modes:
- isocratic: constant eluent composition
- gradient: continuous variation of eluent mixture during analysis

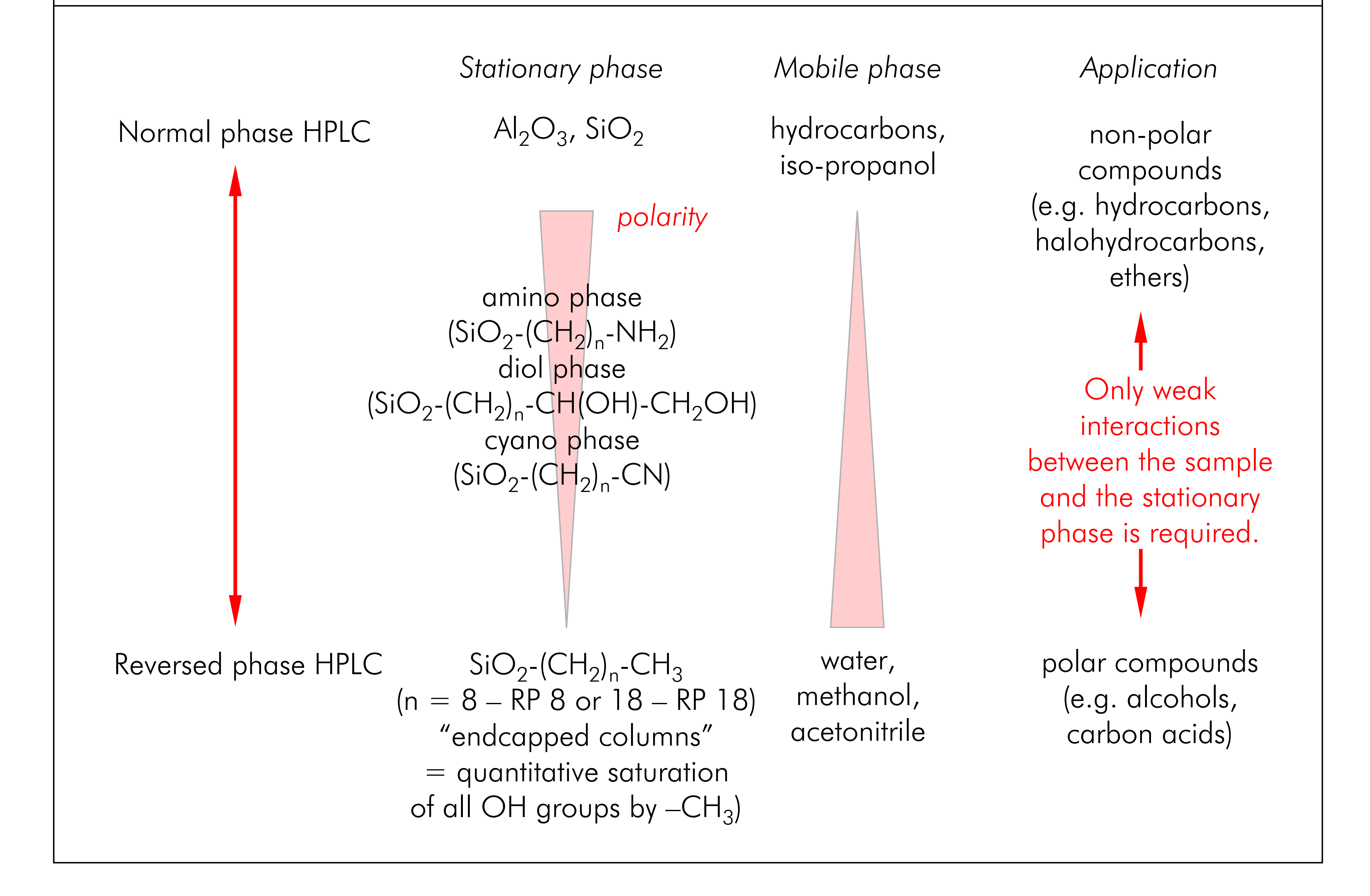
HPLC Injectors

6 port injection valve
(similar to gas sampling valves in GC)



→ Combination with autosamplers for high reproducible sample injection

HPLC Columns



HPLC Detectors

Name	*Selectivity*	*Typical minimum detectable level [g/ml]*	*Linear dynamic range*
UV-VIS detector/ Diode array detector[1]	- for larger organic molecules and transition metal compound which absorb UV-VIS light - time resolved recording of UV-VIS spectra, possibility of deconvaluation of non-separated peaks	$5 \cdot 10^{-10}$	$5 \cdot 10^{4}$
Fluorescence detector[1]	- detects fluorescence radiation emitted by the sample compounds - specific for highly condensed organic molecules like PAH	$10^{-10} \ldots 10^{-9}$	$\sim 10^{3}$
Refraction index detector	- non-specific low-cost detector	$5 \cdot 10^{-10}$	10^{4}
Electric conductivity detector	- specific low-cost detector for compounds dissociated into ions (e.g. inorganic and organic salts, tensides, amino acids)	10^{-8}	10^{3}
Mass selective detector[1]	- most selective detector for HPLC - strong requirements for the interface (transition from the high column pressure to vacuum inside the MSD) - high costs	no data available	10^{5}

1 – suitable for gradient techniques

Special HPLC Techniques

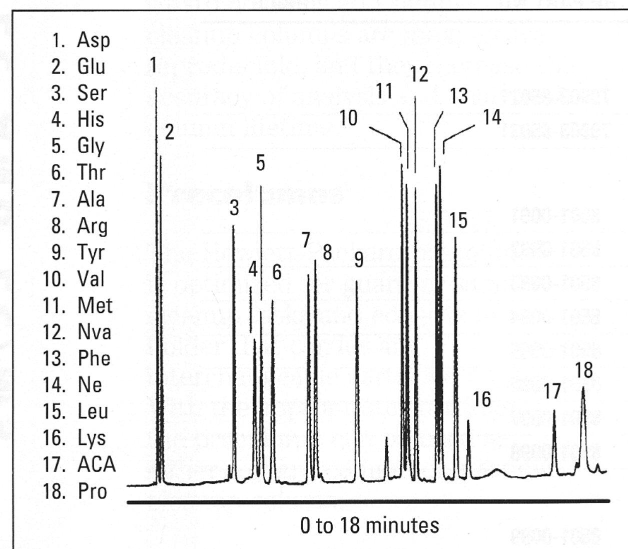
Ion Chromatography/Ion Exchange Chromatography (IC/IEC):

- stationary phase: ion exchange resins
(R-SO_3^- or R-COO^- for cation analysis, R-NH_3^+ or R-N(alkyl)$_3^+$ for anion analysis)
- mobile phase: aqueous solutions
(diluted mineral acids for cation analysis,
hydrogen carbonate buffer for anion analysis)
- detector: electric conductivity detector with pre-installed suppression column
- application: fast analysis of inorganic and organic salts in water
(mainly alkali and alkaline earth metal salts)
- detection limit: 0.5 ppm

Capillary Electrophoresis (CE):

- operation of the column in an electrical field
- Movement of the ions is driven by electrical attraction.
- coupling with typical HPLC detectors (incl. MSD)

HPLC Application Examples

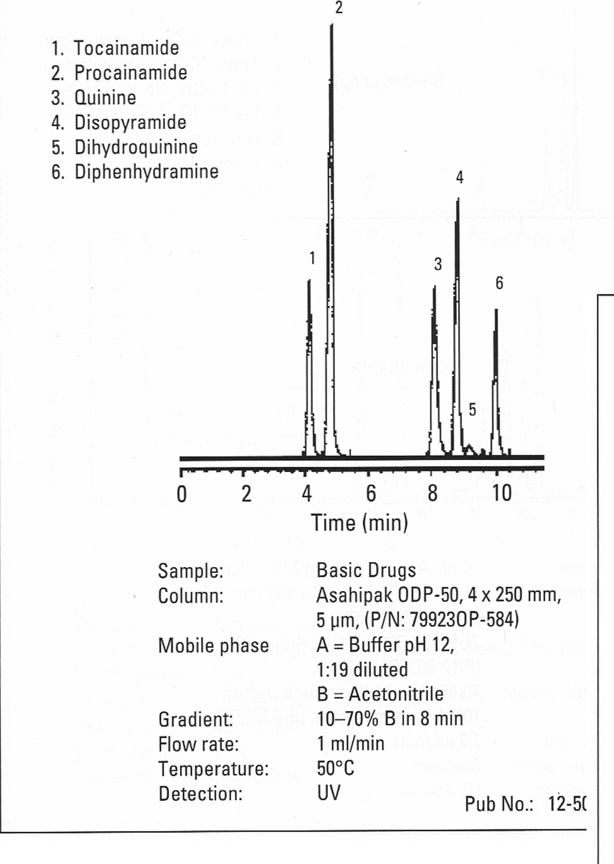


0 to 18 minutes

Sample:	Amino acids (10 pmol each), AminoQuant derivatization
Column:	Amino Acid (C18) column, 2.1 x 200 mm, 5 µm, (P/N: 79915AA-572)
Mobile phase:	A = 20 mM sodium acetate, 0.018% TEA, pH 7.2, 0.3% THF B = 100 mM sodium acetate, pH 7.2, acetonitrile, methanol (1/2/2)
Gradient:	0 to 60% B in 17 min
Flow rate:	0.45 mL/min
Temperature:	40°C
Detection:	Fluorescence

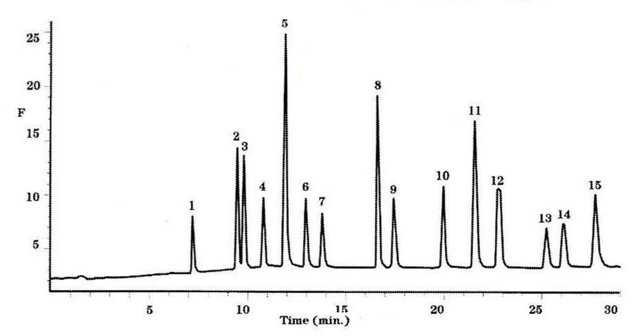
Biotechnological analysis:
Amino acids

Pharmaceutical analysis:
Basic drugs



Environmental analysis:
Polycyclic
aromatic hydrocarbons

1. Napthalene
2. Acenaphtalene
3. Fluorene
4. Phenanthrene
5. Anthracene
6. Fluoranthene
7. Pyrene
8. Benzo(a)anthracene
9. Chrysene
10. Benzo(a)fluoranthene
11. Benzo(k)fluoranthene
12. Benzo(a)pyrene
13. Dibenzo(a,h)anthracene
14. Benzo(g,h,i)perylene
15. Indeno(1,2,3-cd)pyrene



Sample:	PAH Standard
Column:	LiChrospher PAH, 3.0 X 250 mm, 5 µm, (P/N: 79925PA-583)
Mobile phase:	A = Water, B = Acetonitrile
Gradient:	0 min 50% B, 3 min 60% B, 15.4 min 100% B, 23.5 min 50% B
Flow rate:	0.8 mL/min
Temperature:	27°C
Detection:	254 nm

Quantitative Analysis

External standard

(1) Calibration for a known substance by injection of different known substance concentrations, calculation of a regression curve

(2) Injection of the sample with the unknown concentration, back calculation of the concentration by using the calibration function

Internal standard (elimination of sensitivity variations)

(1) Adding of a known equal amount of a substance which is not a part of the sample to each calibration sample and to the sample with the unknown concentration

(2) Normalization of the signal response (peak area or height) of all components on base of a constant signal of the external standard compound ($f = Y_{norm}/Y_{real}$)

Standard addition

(1) Injection of the sample with the unknown concentration

(2) Adding of known amounts of the substance which should be analyzed and performing a new analysis after each addition

(3) Calculation of the unknown concentration by setting $Y = 0$ for the concentration-response-function